

# The Factors Affected to the Spread Dynamics of the Infection by *Echinococcus Granulosus* Hydatid Cyst in Sheep and Goats

Aygun A. Azizova<sup>1\*</sup> and Sevda M. Muradova<sup>2</sup>

<sup>1</sup>Veterinary Scientific Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan,

<sup>2</sup>Azerbaijan State Pedagogical University, Baku c.

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The researches were conducted in order to determine the spreading of echinococcosis in sheep and goats in the Mountainous-Shirvan and Shirvan-Salyan economic regions of Azerbaijan. The liver, lungs, spleen and kidneys of 1200 sheep, 1080 goats from the Mountainous-Shirvan economic region, 1400 sheep and 765 goats from the Shirvan-Salyan economic region used for slaughter from farms, were examined according to the echinococcus cysts. In general, 2,600 sheep from two economic regions were researched on the seasons for 1 year, and were noted in 1,134 animals hydatid cysts (43.6%). In sheep, the infection percentage was high in winter (58.7%) and autumn (56.4%), and low in spring (21.8%). During the year, the hydatid cysts were noted in 796 goats (43.1%) during the examination of 1845 goats. In goats, the infection percentage was noted high in winter (47.6%) and summer (45.1%), and relatively weak in spring (34.3%), regardless of the age dynamics. It was determined that there are invasion protoscoleces in the cysts of 90% of sheep and 85% of goats. In order to determine the infection status of *E.granulosus* cestodes in the main hosts, coprological samples of 82 shepherd dogs, 447 stray dogs, 75 foxes and 39 jackals were examined coprologically. The eggs of *Echinococcus granulosus* cestodes were followed in the samples belonging to 4 shepherd dogs (4.9%), 98 stray dogs (21.9%), 28 foxes (37.3%) and 7 jackals (17.9%). Coprological samples of the main hosts were examined using the methods of Sherbovich, Vishniauskas, and Fulleborn. To detect echinococcus cysts in intermediate hosts, the method of complete dissection was used. Hematoxylin-eosin staining was used to determine the activity of protocols in hydatid cysts. The number of studied animals, the number of hydatid cysts in sick animals, and the average relative coefficient of the number were calculated based on the "Student t criterion".

**Keywords:** Coprological Samples; Echinococcosis; Hematoxylin-Eosin; Hydatid Cyst; Protoscolex; Larval Cestodes.

The rapid growth of the world's population causes the problems such as lack of water, food and oxygen. The most important of these problems is the lack of food worried the whole world. According to statistics, in underdeveloped and developed countries, the population growth is higher than the increasing of the animal products production<sup>1,2</sup>. The development of the animal husbandry

provided the population's meat needs is one of the priority tasks of the agriculture. The special attention is given to these problems in the Republic of Azerbaijan, too. A plan of measures is implemented in the direction of the restoring the species number of the local animal breeds, increasing the productivity of some breeds and increasing the quality of meat and dairy breeds. The invasion

\*Corresponding author E-mail: [azizova\\_aygun@inbox.ru](mailto:azizova_aygun@inbox.ru)



diseases occupy an important place among the important factors influenced to the development of the animal husbandry. The most important of them is hydatidosis caused by *Echinococcus granulosus* cestode, which has a zoonotic complicated life cycle chain<sup>3,4,5</sup>. The adult individuals of the causative agent of hydatidosis or echinococcosis parasitize in the small intestines of dogs, wolves, jackals, foxes and the other Caniformias. The larval stage of the *Echinococcus granulosus* cestode causes an important helminthozoonosis created the hydatid cysts, which localizes mainly in the parenchymatous organs of the intermediate hosts - sheep, goats, cattle and humans. The disease observed in many countries of the world is more met especially in the regions where sheep farming is developed<sup>6,7</sup>. Hydatidosis (echinococcosis), a very common parasitic disease, is distributed on all the continents except Antarctica, has a wide range of primary and intermediate hosts<sup>8</sup>. It is mentioned in the literature that the dogs play an important role in the spread of the disease. However, the spread of the disease is influenced many factors, such as the climate of the region, the keeping conditions of the agricultural animals and the living standard of the people, which is an anthropogenic factor<sup>9</sup>. Therefore, the prevalence rate of the disease appears at different rates in different geographic regions or countries.

The fight against hydatidosis, caused great economic losses and posed a serious threat to the health of the population, the elimination of one factor in the biological cycle of the parasite (preventing the feeding of the infected organs to dogs and the other wild mammals) is of great importance<sup>10,11</sup>. The current problem is that it is impossible to diagnose the hydatidosis due to the absence of the significant clinical signs in the intermediate host animals - sheep, goats and cattle. Although the ultrasound and PCR examinations are used for the diagnosis, they are not practical and efficient for the big farms<sup>12</sup>. In the intermediate host animals, the easiest and most accurate diagnosis of the disease is given based on the autopsy examinations after the slaughter. That's why, the results of the examinations conducted at the slaughterhouses allow to assess the situation related to the disease and its epidemiology<sup>13</sup>.

Since Azerbaijan is a place where livestock farming is developed, it is impossible not to become

infected with echinococcosis in both farm animals and people. According to the statistical data of the recent years, in the country, the intensification of the infection cases not only among the agricultural animals, but also among the population, especially among the women<sup>14</sup>. The mentioned facts of intensification indicate the importance of regular research of this disease in intermediate and main hosts. For the first time, the research was carried out by us on summer and winter grazing habitats of small ruminants in the Mountainous-Shirvan and Shirvan-Salyan economic regions in the direction of the researching the factors affecting the spread of echinococcosis.

## MATERIALS AND METHODS

The livers, lungs, spleen and kidneys of 2,600 sheep and 1,845 goats were examined according to the hydatid cysts of *E. granulosus* cestodes in the researches carried out from February 2022 to March 2023 in the livestock farms of the Mountainous-Shirvan and Shirvan-Salyan economic regions. The pathological materials were taken from the animals used for meat slaughter, as well as from the animals that died in the farms. The fecal samples belonging to 82 shepherd dogs, 447 stray dogs, 75 foxes and 39 jackals, which are the main hosts of the adult individuals of echinococcal cysts, were examined coprologically. Coprological samples of the main hosts were examined using the methods of Sherbovich, Vishniauskas, and Fulleborn. To detect echinococcus cysts in intermediate hosts, the method of complete dissection was used. The hematoxylin-eosin staining was used to determine the activity of the protoscolexes in the hydatid cysts. The age, sex of the animal, the spread dynamics of the disease on the seasons were taken into account in the conducting of the researches. The number of the studied animals, the number of the hydatid cysts in sick animals, the average relative coefficient of the number were calculated according to "Student's t criterion"<sup>15</sup>.

## RESULTS AND DISCUSSION

4445 small ruminants (2600 sheep, 1845 goats) from two economic regions were researched. The hydatid cysts were detected in the liver and

lungs of 1134 (43.6%) of 2600 sheep examined during the research period. In sheep, the hydatid cysts were detected mainly in the liver, and in goats, in the lungs. The hydatid cysts were determined in the liver and lungs of 841 (45.6%) of 1845 goats examined during the investigation (Table 1). Infection with hydatid cyst was recorded in the liver of 864 sheep (76.2%), lungs of 270 sheep (23.8%). Infection with hydatid cysts was noted in the lungs of 515 goats (61.2%) and in the liver of 326 goats (38.8%).

In the results of the examinations, there were no serious differences in the infection extensiveness of sheep and goats with the hydatid cysts ( $X^2: 2.773, DF: 1, p=0.078$ ).

The infection status of small ruminants with the hydatid cyst was researched in the spring,

summer, autumn and winter seasons. The hydatid cysts were detected, in autumn 421 (56.4%) out of 746 sheep, in winter 260 (58.7%) out of 443, in spring 143 (21.8%) out of 656, in summer 310 (41.1%) out of 755 sheep. In sheep the highest infection percentage was observed in the animals slaughtered in the winter season (58.7%). The weakest infection was 21.8% in sheep in the spring season (Table 2).

In order to determine the infection state of goats with the hydatid cysts on the seasons, the infection was detected in 269 (44.8%) out of 600 goats researched in autumn, in 214 (47.6%) out of 450 researched in winter, in 144 (34.3%) out of 420 researched in spring, 169 (45.1%) of 375 goats researched in summer. The highest infection in goats was determined in animals slaughtered

**Table 1.** The infection status of small ruminants with the hydatid cyst

	The number of the examined animals	The number of the infected animals	The extensiveness of the invasion (%)
Sheep	2600	1134	%43.6a
Goat	1845	841	%45.6a
Total	4445	1975	%44.4a

**Table 2.** The infection dynamics of sheep with the hydatid cysts on the seasons

	The number of the examined animals	The number of the infected animals	The extensiveness of the invasion (%)
Autumn	746	421	%56.4a
Winter	443	260	%58.7a
Spring	656	143	%21.8c
Summer	755	310	%41.1b

$X^2:3615.23, DF:3, p<0.05, a,b,c:$  The difference between the groups with the different letters in the same column is important

**Table 3.** The infection dynamics of goats with the hydatid cysts on the seasons

	The number of the examined animals	The number of the infected animals	The extensiveness of the invasion (%)
Autumn	600	269	%44.8a
Winter	450	214	%47.6a
Spring	420	144	%34.3b
Summer	375	169	%45.1a

$2:3615.23, DF:3, p<0.05, a,b:$  The difference between the groups with the different letters in the same column is important

**Table 4.** The age dynamics of the infection of sheep with the hydatid cysts

	The number of the examined animals	The number of the infected animals	The extensiveness of the invasion (%)
up to 1 year old	855	445	%52.0a
1-2 years old	996	406	%40.8b
over 2 years old	749	283	%37.8c

X2:3615.23,DF:3,p<0.05,a,b,c:The difference between the groups with the different letters in the same column is important

**Table 5.** The age dynamics of the infection of goats with the hydatid cysts

	The number of the examined animals	The number of the infected animals	The extensiveness of the invasion (%)
up to 1 year old	871	303	%34.8c
1-2 years old	700	368	%52.6b
over 2 years old	274	170	%62.0a

X2:3615.23,DF:3,p<0.05,a,b,c:The difference between the groups with the different letters in the same column is important

in winter (47.6%). The weakest infection was observed in spring and was 34.3%. In sheep and goats, the infection state with the hydatid cysts on the seasons, has given similar results.

In sheep and goats, the analysis of the infection with the hydatid cysts on the months showed different results. The highest infection in lambs was followed in July-August, in 1-2-year-old sheep in September-October, and in sheep over 2 years old in January-February. The weakest infection in lambs was observed in March-June, in 1-2-year-old sheep in July-August, and in sheep over 2 years old in April-June. In goatlings the infection on all the seasons showed statistically similar results, and the invasion was evaluated weakly. In 1-2-year-old goats, the highest infection was found in November-February, and the lowest infection was met in the April-September months. In goats over 2 years of age, the highest infection was determined in November-December, and the lowest infection was determined in March-April. Although the infection with the hydatid cysts in sheep showed similar results throughout the year, but the infection in goats had a fluctuating dynamic.

The situation of infection with hydatid cysts of sheep and goats studied in two economic regions was analyzed according to different age

groups. For this purpose, the studied sheep and goats were divided into age groups up to 1 year old, from 1 year old to 2 years old and over 2 years old. The highest infection of the liver and lung with the hydatid cysts was noted in lambs under 1 year old (52.0%), relatively weak in 1-2 year old sheep (40.8%), the lowest infection was noted in older sheep older than 2 years (37.8%) ( Table 4).

In the researched goats on the age groups, the highest infection was observed in animals over 2 years old (62.0%), relatively weak in 1-2 year old goats (52.6%), the lowest infection was observed in goats up to 1 year old (34.8%) ( Table 5.). In contrast to sheep, the infection with the hydatid cysts in goats showed higher results in animals older than 2 years.

The statistical results of the hydatid cysts found in the organs of sheep of different age groups and the statistical results of the average relative coefficient of the number of the protoscoleces in these hydatid cysts (1 ml) were different.  $38.7 \pm 2.88$  hydatid cysts/animal and  $4.06 \pm 0.69$  thousand protoscolex were followed in lambs under 1 year of age,  $29.3 \pm 1.89$  hydatid cysts/animal and  $2.58 \pm 0.26$  thousand protoscolex in sheep from 1 to 2 years of age,  $21.5 \pm 2.17$  hydatid cysts/animal and  $2.92 \pm 1.17$  thousand protoscolex in sheep older

than 2 years, respectively. As can be seen from the results, the average relative coefficient was higher in lambs under 1 year of age.

The statistical results of the hydatid cysts found in the organs of goats of different age groups and the statistical results of the average relative coefficient of the number of the protoscolexes in these hydatid cysts (1 ml):  $9.4 \pm 0.43$  hydatid cysts/animal and  $0.43 \pm 0.036$  thousand protoscolexes were followed in goatlings up to 1 year of age,  $11.4 \pm 0.66$  hydatid cysts/animal and  $0.63 \pm 0.069$  thousand protoscolex in goats from 1 to 2 years old,  $31.2 \pm 2.14$  hydatid cysts/animal,  $2.51 \pm 0.43$  thousand protoscolex in goats over 2 years old. As can be seen from the results, the average relative coefficient was higher in goats older than 2 years.

The fecal samples belonging to 82 shepherd dogs, 447 stray dogs, 75 foxes and 39 jackals were examined in order to detect the adult individuals of the *Echinococcus granulosus* cestode and determine the role of shepherd dogs and the other wild dogs, which are the main hosts in the spread of echinococcosis. The intensive detected parasites in shepherd dogs were *Toxocara canis* (45/54,9%), *Ancylostoma caninum* (38/46,3%), *Trichuris vulpis* (32/39,0%), in stray dogs were *Toxocara canis* (350/78,3%), *Ancylostoma caninum* (300/67,1%), *Dipilidium caninum* (225/50,3%), *Strongyloides sp.* (156/34,9%), *Isospora canis* (175/39,1%), *Sarcocystis sp.* (280/62,6%), *Toxoplasma gondii* (196/43,8%), but only in 4 shepherd dogs (4.9%), in 98 stray dogs (21.9%), the *Echinococcus granulosus* cestode eggs were followed. The *Alaria alata* (51/68,0%), *Trichuris vulpis* (43/57,3%), *Angiostrongylus sp.* (38/50,7%) parasitic eggs were observed intensively in the researched foxes, but the *Echinococcus granulosus* eggs were detected in only 28 foxes (37.3%) out of 75. The *Alaria alata* (25/64,1%), *Pharyngostomum sp.* (21/53,8%) trematodes, *Toxocara sp.* (31/79,5%) nematode eggs were observed intensively in the researched jackals, but the *Echinococcus granulosus* eggs were detected in only 7 jackals (17.9%) out of 39.

## DISCUSSION

In the researches conducted in different countries, the prevalence of the hydatidosis in sheep was detected 20.3% in Spain, 70-92.8% in the Sardinian region and 5-28% in the Sicilian region

of Italy, up to 80% in Greece<sup>16,17,18</sup>. Researcher noted that the infection rate in sheep in Libya varies from 1.6-40%, and in goats from 5.6-70%<sup>19</sup>. It is noted that the prevalence of *E. granulosus* is quite high in Tunisia<sup>20</sup>. In the another North African country, Algeria, the infection rate with echinococcosis in sheep was noted 2.24%<sup>21</sup>. In Beirut, it was determined that 62.9% of sheep and 20.9% of goats had a positive result according to echinococcosis from 2018 to 2020<sup>22</sup>. In the Kangra Valley of the Himalayas in North-Western India, the prevalence intensity of the hydatid cysts in the various organs of small ruminants was researched. The hydatid cysts were detected in 12.2% of sheep and 10.7% of goats<sup>23</sup>.

In the researches conducted in the East Azerbaijan region of Iran, the highest percentage of the patients with the hydatid cysts were women (56.2%). The lowest infection was noted in 2012 (11.4%), and the highest infection in 2015 (17.0%). The Infection with echinococcosis was detected mostly in the rural population (62.2%)<sup>24</sup>. In the researches conducted on small ruminants in Iran, the hydatid cysts were detected in 546 (45.5%) of 1200 slaughtered sheep at slaughterhouses<sup>25</sup>. The other researchers determined the infection rate of the lungs and livers infected with the hydatid cysts in sheep and goats in the Iran territory. It was determined that in sheep, 15.28% of the lung and 6.08% of the liver were infected with the hydatid cysts, and in goats, respectively 19.15% of the lungs and 10.28% of the liver were infected with the hydatid cysts<sup>26</sup>.

In different regions of Turkey, in the researches conducted by different researchers, the different results have been reported regarding to the prevalence of the hydatidosis. In the Burdur province, the prevalence of the invasion was 26.6% in sheep, in K yr kkale it was 3.2% in lambs, and 50.9% in sheep<sup>27,28</sup>. The prevalence of the hydatid cysts in sheep was 46.6% in Van, 63.85% in Kars, and 35.7% in Sivas<sup>29,30</sup>. In the researches conducted in Konya, the hydatid cysts were detected in the livers of 5.64% of sheep and 1.68% of lambs. The economic losses caused as a result of the liver destruction, were calculated taking into account the sales prices in 2019, and it was calculated that a total of 36,450 TL (6,417 US dollars) of financial loss occurred due to the destruction of 810 livers<sup>31</sup>. In the researches conducted in Turkey, it was

used from the GenBank nucleotide sequence data and it was determined that found that the hydatid cysts detected in sheep and cattle have the same haplotypes as humans. It has been noted that more longer nucleotide sequences and more larger human sample sets must be used for to determine the biological significance of the *E.granulosus* haplotypes. The conducting of the researches with this method will allow to study the genetic variability of *E.granulosus* in sick people<sup>32</sup>.

In the Caspian Sea region of Russia, in sheep, it is noted that the infection with the *Echinococcus granulosus* cysts increases 0.2-0.3% every year and is 32.8% on average. It has been shown that in the natural altitude zones, in shepherd dogs, the intensity of the *Echinococcus granulosus* invasion is  $3136.7 \pm 343.0$  parasites/animal on average and the extensiveness is 30.1%, respectively<sup>33</sup>.

Research works in this direction are also being conducted in the Republic of Azerbaijan. The experiments show that as the intensity of the distribution of the *E.granulosus* cestode, which has a complex chain life circulation, increases in the main hosts, in addition to farm animals, which are intermediate hosts, the infection is increasing in humans, too. As in the whole world, in Azerbaijan, it was not possible to reduce the prevalence rate of echinococcosis among the people. The research results show that the intensity of the infection in the main hosts, which play an important role in the spread of echinococcosis, has caused to the increase of the disease cases in humans in the recent years. It has been determined that the disease has intensified in women in the recent years. 96 were men ( $35.3 \pm 2.90\%$ ), 176 were women ( $64.7 \pm 2.90\%$ ) of the 272 examined patients. The occurrence frequency of the disease between men and women was in the ratio of 1:2, the majority of the patients were between 18-60 years old (238 patients, 87.5%)<sup>34</sup>. In the researches conducted in some regions of Azerbaijan (during 2001-2017), it was determined that the *E.granulosus* cestode spread from 17.7% to 28.8% among the stray dogs<sup>35</sup>.

In the Mountainous-Shirvan and Shirvan-Salyan economic regions, where livestock farming is widespread, high rates of small animal echinococcus infection were recorded. Comparing the two economic regions, the results were higher in Mountainous-Shirvan. In Mountainous-Shirvan,

54.9% of cases of *E.granulosus* cestode infection were recorded in sheep and 56.6% in goats. In this economic region, the degree of infection of sheep and goats with echinococcal cysts was estimated at high and similar rates. In the Shirvan-Salyan economic region, 35.3% of *E.granulosus* cestode infection was recorded in sheep and 31.3% in goats. The results show that echinococcal infection of sheep and goats is higher in the Mountainous-Shirvan economic region compared to the Shirvan-Salyan region. According to the research results, it is known that foxes are the main hosts of the cestode *E.granulosus* in the Republic of Azerbaijan. The natural focus of the disease is forest areas inhabited by wild animals. In recent years, climate change, the use of forest areas as pastures and the change of biotopes caused by the search for food by animals have directly led to the urbanization of wild animals in villages and cities. In addition to these factors, the well-known war in the regions close to the forest areas of the country has led to the intensive appearance of wild animals, especially foxes, even in the morning in villages and towns. *E.granulosus* eggs spread by foxes caused intensive infection of small horned animals with hydatid cysts and a local outbreak of echinococcosis. This confirms the importance of protecting animals from contact with wild animals, especially foxes, which are more important than stray and shepherd dogs in the prevention of echinococcosis in small horned animals. As a result of our research, it was established that in the study regions, the most common definitive hosts of *E.granulosus* are foxes. The natural focus of the disease is the forest areas inhabited by wild animals. In the recent years, the climate change, the use of the forest areas as grazing land and the biotope change caused according to the search of food by animals, caused directly to the urbanization of the wild animals to the villages and towns. In addition to these factors, the known war in the regions near to the forest-rich areas of the country has caused to the intense appearance of the wild animals, especially foxes, even in the morning hours in the villages and towns. The eggs of *E.granulosus* spread by foxes caused the intensive infection of small ruminants with the hydatid cysts and the local outbreak of echinococcosis. And this confirms that it is important to protect the animals from contact with the wild animals, especially

foxes, which are more important than stray and shepherd dogs in the prevention of echinococcosis in small ruminants.

### CONCLUSION

Analysis of the conducted studies shows that infection with *E. granulosus* larvae is intensively observed in sheep and goats. Of 1100 examined sheep in Mountainous-Shirvan, 604 were infected with the cestode *E. granulosus* (54.9%). Of 1045 examined goats in this economic region, infection was detected in 591 (56.6%). In the Shirvan-Salyan economic region, 530 (35.3%) of 1500 sheep were infected. Of 800 goats examined in this economic region, 250 (31.3%) were infected. The extensiveness of infection with echinococcal cysts in sheep and goats was estimated at high and similar rates. This indicates an increase in the intensity of the spread of cestodes in the main hosts - dogs, foxes, jackals. The current situation confirms the increased risk of infection of humans, as well as farm animals, which are intermediate hosts of echinococcosis. To reduce the risk of spreading echinococcosis, which has epidemiological significance, it is necessary first of all to exclude farm animals from natural foci of echinococcosis. This includes not using forest areas with dense wildlife habitat as pastures, and not grazing animals on pastures adjacent to forests. Disinfection of organs of sick animals is also an important condition for disease prevention. One of the main conditions for the fight against echinococcosis is the timely detection of sick animals on farms and their use for slaughter. To prevent infection on farms, it is necessary to carry out comprehensive veterinary and sanitary measures. Shepherd dogs, which play the main role of the owner in the spread of the disease, according to the instructions, should be dewormed 4 times a year.

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### Conflict of Interest

The authors do not have any conflict of interest.

### Data Availability Statement

This statement does not apply to this article.

### Ethics Statement

This research is approved by the Animal ethics committee of the Azerbaijan Scientific Research Institute of Veterinary, Ministry of Agriculture of Azerbaijan Republic

### Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

### Authors' Contribution

Azizova Aygun: Conceived and design the analysis, collected the data, contributed the data, performed the data analysis, and wrote the paper  
Muradova Sevda: Collected the data

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